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Amend B

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Dahm *et al.*

Serial No.: 09/601,645
Confirmation No.: 7793

Filed: August 4, 2000

For: *METHOD FOR THE QUANTITATIVE DETERMINATION OF TUMOR CELLS IN A BODY FLUID AND TEST KITS SUITABLE THEREOF*

Art Unit: 1655

Examiner: Zitomer, S.

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Kelly Fischer
Kelly Fischer

AMENDMENT

Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

Responsive to the Office Action, mailed October 24, 2001, please amend the application as follows:

IN THE SPECIFICATION:

Please amend the specification as follows (a marked-up copy of the amended specification is attached to this Amendment):

At page 1, line 1, insert:

—This application is the National Stage of International Application No. PCT/EP99/00716, filed February 3, 1999. Benefit of priority under 35 U.S.C. §365(b) to German Application No. 198 04 372.4, filed February 4, 1998 is claimed herein.—

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Please replace the paragraph on page 7, line 27 to page 8, line 26, with the following paragraph.

The subsequent amplification can be carried out, for example, with DNA polymerase, for example by the polymerase chain reaction (PCR) (see, for example, U.S. Patent Nos. 4,683,195; 4,683,202; 4,965,188) or, preferably, with an RNA polymerase by, for example, isothermal nucleic acid sequence-based amplification (NASBA). Specific oligonucleotide primers derived from the

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nucleic acid to be amplified are required for the amplification in each case. It is possible in the present invention to use any sequence section of the cDNA coding for the catalytic subunit of telomerase for synthesizing the oligonucleotide primers. The oligonucleotide primers are preferably about 20 to about 40, preferably about 25 to 35, nucleotides long. The amplification product is generally about 100 to about 2000 bases, preferably about 200 to about 1500 bases, in particular about 450 to about 550 bases, long. The following oligonucleotide primers, which have been derived from the sequence shown in Fig. 1, are particularly preferred for the novel method:
5′ CTACCGGAAG AGTGTCTGGA GCAAGTTGCA AAGC 3′ (hTRT1) (SEQ ID NO. 1), and/or
5′ GGCATACCGA CGCACGCAGT ACGTGTTCTG 3′ (hTRT2) (SEQ ID. NO. 2), where hTRT1 and/or hTRT2 may, where appropriate, additionally comprise a promoter sequence for an RNA polymerase. The oligonucleotide primer hTRT1 corresponds to the 5′ primer and hTRT2 corresponds to the 3′ primer. The amplification product is 513 bp long. The primers may, for example, be prepared synthetically using the triester methods (Matteucci et al., (1981), J. Am. Chem. Soc., 103, 3185-3191). The DNA polymerase which can be used is, for example, a non-thermostable DNA polymerase such as T4 DNA polymerase, T7 DNA polymerase, E. coli polymerase I or the Klenow fragment of E. coli or, preferably, a thermostable DNA polymerase such as Taq polymerase (see, for example, U.S. Patent No. 4,889,818).

Please replace the paragraph on page 14, lines 27-39 with the following paragraph.

As internal positive control of the method and of the sample to be investigated it is possible additionally to amplify and detect a nucleic acid which generally always occurs in a body fluid. Examples of suitable nucleic acids are the mRNA coding for β-globin or for glyceraldehyde-phosphate dehydrogenase (GAPDH) (see, for example, GB 2 260 811) which always occur in the cells of

the body fluid. Suitable oligonucleotide primers for human β-globin mRNA are, for example, primers with the sequences:

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5′ ACCCAGAGGT TCTTTGAGTC 3′ (Glob 1) (SEQ ID. NO. 3) and

5′ TCTGATAGGC AGCCTGCACT 3′ (Glob 2) (SEQ ID. NO. 4)

Please replace the paragraph on page 15, lines 1-22 with the following paragraph.

Further internal positive controls of the method and of the sample to be investigated can additionally be cell-specific nucleic acids, such as β-actin mRNA, with the primers (Nakajima-Iiji, S., Hamada, H., Reddy, P., Kakanuga, T. (1985): Molecular structure of the cytoplasmatic β-actin gene: Interspecies homology of sequences in the introns. Proc Natl Acad Sci USA 82, 6133-7):

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5′ GATGATGATATCGCCGCGCTCGTC 3′ (Act 1) (SEQ ID. NO. 5)

5′ CTCAAACATGATCTGGGTCATCTTC 3′ (Act 2) (SEQ ID. NO. 6)

or T-cell-specific nucleic acids, such as the mRNA of the T-cell receptor, with the primers (Toyonaga, B., Yoshikai, Y., Vadasz, V., Chin, B., Mak, T.W. (1985): Organization and sequences of the diversity, joining, and constant region of the human T-cell receptor β chain. Proc Natl Acad Sci USA 82, 8624-8):

5′ GAGGTCGCTGTGTTTGAGCCATCAGAAG 3′ (TCR 1) (SEQ ID. NO. 7)

5′ GATCTCATAGAGGATGGTGGCAGACAG 3′ (TCR 2) (SEQ ID. NO. 8)

Please replace the paragraph on page 26, line 25 to page 27, line 3 with the following paragraph.

The present invention further relates to the oligonucleotide primers with the sequence

5′ CTACCGGAAG AGTGTCTGGA GCAAGTTGCA AAGC 3′ (hTRT1)

(SEQ ID. NO. 1) and/or

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5′ GGCATACCGA CGCACGCAGT ACGTGTTCTG 3′ (hTRT2) (SEQ ID. NO. 2), where hTRT1 and/or hTRT2 may, where appropriate, additionally comprise a promoter sequence for an RNA polymerase;

and an oligonucleotide with the sequence

5' CGTTCTGGCT CCCACGACGT AGTC 3' (hTRT o) (SEQ ID. NO. 9)
and the corresponding reverse complementary sequences of the oligonucleotide for detecting the amplified "antisense" RNA.

Please replace the paragraph on page 27, lines 4-20 with the following paragraph.

The invention additionally relates to a kit for quantifying tumor cells in a body fluid, for example blood, urine or else stool, exudates or transudates from body cavities, especially peripheral blood, comprising

(a) oligonucleotide primer pair for specific amplification of telomerase-encoding nucleic acid, where the oligonucleotide primer pair preferably has the following sequences:
5' CTACCGGAAG AGTGTCTGGA GCAAGTTGCA AAGC 3' (hTRT1)
(SEQ ID. NO. 1) and/or
5' GGCATACCGA CGCACGCAGT ACGTGTTCTG 3' (hTRT2) (SEQ. ID. NO. 2),
where hTRT1 and/or hTRT2 may, where appropriate, additionally comprise a promoter sequence for an RNA polymerase.

Please replace the paragraph on page 28, lines 27-24 with the following paragraph.

Fig. 1 shows the sequence described by Nakamura et al., (SEQ ID. NO. 10) encoding the catalytic subunit of human telomerase, of 4015 base pairs (bp), and the position of the designed oligonucleotide primers or the oligonucleotide probe (hTRT o) (SEQ ID. NO. 9; underlined): 5' primer hTRT1 (position 1780-1813) (SEQ ID. NO. 1), 3' primer hTRT2 (position 2261-2290) (SEQ ID. NO. 2) with an amplification product of 513 base pairs (bp) and the probe hTRT o (position 1964-1987) (SEQ ID. NO. 9).

Please replace the paragraph on page 35, lines 14-28 with the following paragraph.

The two oligonucleotide primers:
5' CTACCGGAAG AGTGTCTGGA GCAAGTTGCA AAGC 3' (hTRT1)
(SEQ. ID. NO. 1) and
5' GGCATACCGA CGCACGCAGT ACGTGTTCTG 3' (hTRT2) (SEQ ID. NO. 2)

were designed in accordance with the sequence, published by Nakamura et al., coding for the catalytic subunit of human telomerase (Nakamura et al. (1997). Science 277: 955-9) (Fig. 1 and SEQ ID. NO. 10) and synthesized using an Applied Biosystem 380A synthesizer. The specificity of the hTRT1 and hTRT2 primers was checked by computer-assisted analysis of homology on the nucleic acid sequences in the GenBank, EMBL, DDBJ and PDB databases using BLASTIN 1.4.9 MP (Altschul, S.F. et al. (1990), J Mol Biol 215: 403-410].

Please replace the paragraph on page 37, line 3 to page 38, line 18 with the following paragraph.

The construct pGEM-hTRT is created as initial construct for the constructs pGEM-hTRT(Ka), pGEM-hTRT(Kb) and pGEM-hTRT(Kc). pGEM-hTRT(Ka) pGEM-hTRT(Kb) and pGEM-hTRT(Kc) differ from pGEM-hTRT and from one another by a randomized exchange of sequence of about 20 base pairs (bp). The constructs are used for in vitro transcription with Sp6 RNA polymerase of the standard RNA: hTRT(Ka), hTRT(Kb) and hTRT(Kc). To form the construct pGEM-hTRT, the cDNA of the catalytic subunit of human telomerase (Fig. 1 and SEQ ID. NO. 10) is cloned, for example, into the NotI and HindIII cleavage sites of pGEM-13Zf(+). This is achieved by carrying out an RT-PCR with these cleavage-site-containing oligonucleotide primers, which are derived from the sequence hTRT (Fig. 1 and SEQ ID. NO. 10), on the previously isolated RNA from tumor cells or lines under the conditions described above. Thus, it is possible, for example, to amplify the full-length hTRT with given cleavage sites, or a shorter fragment. After a restriction hydrolysis with specific restriction enzymes, for example NotI and HindIII, the fragment formed is cloned into the corresponding cleavage sites (for example position 12 or 38) of pGEM-13Zf(+) and the construct pGEM-hTRT is created. pGEM-hTRT(Ka) is constructed by replacing an about 20bp sequence in the construct pGEM-hTRT is created. pGEM-hTRT(Ka) is constructed by replacing an about 20bp sequence in the construct pGEM-hTRT by an about 20bp cassette. This replacement is carried out by recombinant PCR and is a modification of the method described by

Higuchi et al. [Higuchi, R. (1988). Nucleic Acid Res 16: 7351-7367; Higuchi, R. (1990). M. Innis A. et al. Eds. San Diego, New York, Berkley, Boston, London, Sydney, Tokyo, Toronto, Academic Press, Inc. 177-183]. In a first step, two independent PCR reactions are carried out on pGEM-hTRT: the amplification product from the 1st PCR gives the 5' fragment and is digested with suitable restriction enzymes to give a 5' fragment. The amplification product from the 2nd PCR reaction gives the 3' fragment and is hydrolyzed with suitable restriction enzymes to give a 3' fragment. Using T4 ligase, the cleavage sites of the 5' and 3' fragments are connected to give a fragment which is cloned into the corresponding cleavage sites of pGEM-13Zf(+), to create the construct pGEM-hTRT(Ka). pGEM-hTRT(Kb) and pGEM-hTRT(Kc) are constructed by replacing the about 20 bp sequence, created above, in the construct pGEM-hTRT(Ka), in each case with a randomized sequence of about 20bp. RNA can then be produced in vitro from pGEM-hTRT(Ka), pGEM-hTRT(Kb) and pGEM-hTRT(Kc) with Sp6 RNA polymerase. The specific RNAs can then be detected with oligonucleotides O(Ka), O(Kb), O(Kc) and W(wt), which are complementary to the abovementioned about 20bp replacement sequences and to the wild-type sequence (wt), respectively. The further processing of the RNA, such as DNAse digestion, purification and calibration, is carried out by standard methods.

IN THE CLAIMS:

Please add the following claims:

69. The method of Claim 20, wherein the tumor cells are separated from telomerase-positive non tumor cells.

70. The method of Claim 20, further comprising, before step (i), adjusting the density of the cell separation medium and thereby separating tumor cells from telomerase-positive non tumor cells.

Please replace claims 1, 20, 24, 29 and 34 with amended claims 1, 20, 24, 29 and 34 as follows:

1. (Amended twice) A method for the quantification of tumor cells in a body fluid, comprising:

(a) concentrating or depleting tumor cells in a sample of a body fluid;

B11 (b) specifically amplifying, from the tumor cells, mRNA coding for the catalytic subunit of telomerase; and

(c) quantitatively determining the amount of amplified nucleic acid, thereby quantifying tumor cells in a body fluid.

20. (Amended twice) The method of Claim 1, wherein step (a) comprises:

B12 (i) covering a cell separation medium with a layer of the body fluid;

(ii) centrifuging the cell separation medium covered with the body fluid; and (iii) collecting the tumor cells at the interface of the cell separation medium and the supernatant body fluid.

24. (Amended twice) The method of Claim 20, wherein the body fluid is blood and prior to applying the body fluid sample to the cell separation

B13 medium, the body fluid is mixed with one or more substances that prevent aggregation of platelets to tumor cells, and/or prior to applying the body fluid sample to the cell separation medium, the body fluid is freed of substances that promote aggregation of platelets to tumor cells.

29. (Amended twice) The method of Claim 20, wherein after

B14 centrifugation and before collecting the tumor-cell-enriched interface, the centrifugation vessel is removed and cooled to prevent mixing of the cells in the different layers.

34. (Amended twice) The method of Claim 20, wherein a dye is added

B15 to color the cell separation medium, whereby the color of the cell separation medium is distinguishable from that of the supernatant body fluid.

REMARKS

A check for the fee for a one month extension of time and two added claims accompanies this response. Any fees that may be due in connection with filing this paper or with this application may be charged to Deposit Account No. 50-1213. If a Petition for Extension of time is needed, this paper is to be considered such Petition.

Claims 1-70 are pending in this application. Claims 1-19, 35-61, 67 and 68, while being withdrawn, are retained in light of the Petition under 37 C.F.R. §1.144, filed December 26, 2001, for removal of the Restriction Requirement in the above-captioned application. Claims 69 and 70 have been added. Basis for Claims 69 and 70 may be found, *e.g.*, at page 15, lines 24-34; page 17, lines 22-38 and page 24, lines 20-27 of the specification.

Claims 1, 20, 24, 29 and 34 are amended to particularly point out the claimed subject matter. Claim 1, which is retained, is amended to particularly point out the subject matter of Claim 20, which depends from Claim 1. The amendments to Claims 1 and 20 find basis in original claims 1 and 20 and in the specification, *e.g.*, at page 5, lines 24-33; page 19, line 30 to page 20, line 1; and page 20, line 39 to page 21, line 4. The amendments to claims 24, 29 and 34 correct minor informalities to particularly point out the subject matter of the claims.

The specification has been amended to refer to the German priority application to which the instant application claims benefit under 35 U.S.C. §365. The specification is also amended to include references to SEQ ID. NOS., in compliance with Sequence Rules. No new matter has been added.

Included as an attachment is a marked-up version of the specification paragraphs and claims that are amended, per 37 CFR §1.121. Also attached are duplicate hard and disk copies of the Sequence Listing originally filed August 4, 2000, in connection with the above-captioned application.

CO-PENDING APPLICATION

The Examiner's attention is directed to related copending application U.S.S.N. 09/890,649 filed August 3, 2001, by Dahm, Michael W., which is the U.S. National Stage of International Application No. PCT/EP00/00831, filed February 2, 2000. A verified English translation of the aforementioned International Application No. PCT/EP00/00831 was provided in an Information Disclosure Statement filed October 8, 2001, in connection with the above-captioned application.

RESTRICTION REQUIREMENT

Error in setting forth Restriction Requirement: Misallocation of claims

The Examiner notes an error in the Restriction Requirement mailed July 3, 2001, in connection with the above-captioned application, stating that Claim 67 was included in Group II whereas it "clearly" belongs in Group I, being dependent from Claim 1. The Examiner has therefore amended Group I to contain claims 1-19, 52-61, 67 and 68, which have been withdrawn, while the Group II claims, which are pending, are now claims 20-66 and 62-66.

In the Restriction Requirement of July 3, 2001, the Examiner grouped the claimed subject matter as being directed to:

Group I: nucleic acid amplification methods

Group II: method and apparatus for concentrating tumor cells

Group III: oligonucleotide and oligonucleotide kit

It appears that the Examiner's sole basis for determining that Claim 67 is now "clearly" a Group I claim lies in its being dependent on Claim 1, which is a Group I claim. Applicant notes, however, that as pointed out in the traversal of the Restriction Requirement mailed August 3, 2001, in connection with the above-captioned application, the Group II claims are also dependent on Claim 1.

All of the pending and withdrawn claims are directed to a method for the quantification of tumor cells in a body fluid comprising the steps of (i) concentrating or depleting the tumor cells from a sample of body fluid; and (ii) specifically amplifying tumor cell mRNA for the catalytic subunit of telomerase. The dependent claims specify particulars of the method. The Examiner, nonetheless, restricted the claims describing particulars of step (i) as being directed to a "method for concentrating tumor cells" (Group II claims) and the claims describing particulars of step (ii) as being directed to a "method for amplification" of tumor cell mRNA (Group I claims). The Examiner also restricted Claim 1, which describes both steps (i) and (ii), as a Group I claim.

Even if the Examiner's basis for setting forth the Restriction Requirement is proper (and such is not acknowledged, *see* below), Claim 67, which specifies

the types of tumor cells that may be analyzed by the claimed methods, could provide a qualifying element to either the "Group I" claims (step (ii); specifying the types of tumor cells whose mRNA for the catalytic subunit of telomerase may be amplified) or the "Group II" claims (step (i); specifying the types of tumor cells that may be concentrated or depleted from a body fluid). Therefore, the Examiner's reasons for re-classification of Claim 67 as a Group I rather than a Group II claim are unclear because (1) although Claim 67 depends from Claim 1, a "Group I" claim, the "Group II" claims, to which Claim 67 was originally assigned, also depend from Claim 1; and (2) the particulars of Claim 67 could qualify either the "Group I" or the "Group II" claims.

Notwithstanding this, Applicant has retained all withdrawn claims, pending consideration of a Petition filed December 26, 2001 (*see* below), for removal of the Restriction Requirement.

Response to Applicant's Traversal

(a) The Examiner was not persuaded by Applicant's traversal mailed August 3, 2001, in response to the Requirement for Restriction mailed July 3, 2001, and has made the Requirement for Restriction Final. The Examiner alleges that, contrary to Applicant's assertion that the claims are directed to methods for quantification of tumor cells in a body fluid, the particulars of Group I, directed to amplifying and quantifying mRNA, are distinct from the Group II particulars of concentrating tumor cells. The Examiner therefore maintains the premise that the single general inventive feature between the two groups is "mRNA encoding the catalytic subunit of human telomerase."

Applicant respectfully disagrees with the Examiner's characterization of the common inventive feature of the claims. Contrary to the Examiner's assertion, the Group I claims and Group II claims are not separated in their being directed to a the allegedly distinct particulars of (i) a method for amplifying and quantifying mRNA, (Group I) and, (ii) a method for concentrating tumor cells (Group II). Rather, the claims are directed to a method for the quantification of

tumor cells in a body fluid, and the "Group I" and "Group II" claims are directed to individual steps of the claimed method.

Briefly, claims of Group II are dependent on claims of Group I, and, hence by virtue of the definition of dependent claims, Group II includes all of the particulars of Group I. Claim 1 of Group I is as follows:

> A method for the quantification of tumor cells in a body fluid, comprising:
> (a) concentrating or depleting tumor cells in a sample of a body fluid;
> (b) specifically amplifying, from the tumor cells, mRNA coding for the catalytic subunit of telomerase; and
> (c) quantitatively determining the amount of amplified nucleic acid, thereby quantifying tumor cells in a body fluid.

Claim 20, classified as belonging to Group II, is directed to the method of claim 1 and specifies particulars of step a). If Claim 1 is deemed allowable, Claim 20, which includes all limitations thereof, must also be deemed allowable.

Further, Applicant maintains (*see* below) that the special technical feature linking the claims is a method for the quantification of tumor cells in a body fluid in which the tumor cells are concentrated and the tumor cell mRNA for the catalytic subunit of telomerase is specifically amplified using particular primers. Furthermore, as discussed below, this special inventive feature is novel and inventive over the cited art.

(b) In the paper mailed August 3, 2001, Applicant argued that neither the Sidransky (U.S. Patent No. 5,726,019) nor the Selby (GB 2 260 811) references taught or suggested anything about telomerase or mRNA coding for the catalytic subunit of telomerase. In response, the Examiner urges that these references were cited as a courtesy to Applicant to show that concentrating cells and amplifying and quantifying mRNA were known techniques in the art, that the issue is restriction, not rejection over prior art, and that the Examiner was only required to provide a reference disclosing the special technical feature.

In the Restriction Requirement set forth on July 3, 2001, the Examiner's characterization of the "mRNA for the catalytic subunit of telomerase" as the

special technical feature, known in the prior art, of both Groups I and II, was based on the following reference:

(A) cDNA sequence from mRNA encoding the catalytic subunit of telomerase, available from Genbank and set forth in Nakamura *et al.*, *Science*, 277:955-959 (1997).

The Examiner provided two additional references, which the Examiner now alleges were cited as a "courtesy":

(B) Sidransky (U.S. Patent No. 5,726,019), which allegedly practised the "claimed [Group I] method of amplifying mRNA from tumor cells in a body fluid by measuring the amount of specific mRNA in the cells".

(C) Selby (GB 2 260 811), which allegedly taught the "claimed" Group I method and also the "claimed [Group II] method of concentrating tumor cells".

It is respectfully submitted that Applicant's response was commensurate with the Examiner's assertions in the Requirement for Restriction set forth on July 3, 2001. Applicant argued that none of the cited references, singly or in combination, taught the special technical feature linking the instant claims, namely, a method for the quantification of tumor cells in a body fluid by specifically amplifying the mRNA for the catalytic subunit of telomerase and the primers/kits used in said method. Applicant is aware that the issue is restriction, and not rejection over the prior art. With that in mind, and in response to the Examiner's allegations, Applicant argued that the special technical feature linking the instant pending and withdrawn claims is (i) a method for the quantification of tumor cells in a body fluid by specifically amplifying the mRNA for the catalytic subunit of telomerase and the primers/kits used in said method; and (ii) that the special technical feature is novel and inventive over the prior art.

U.S. Patent No. 5,726,019 describes a method for diagnosing lung neoplasia and is based upon the discovery that a nucleic acid molecule that has

as particular mutation is associated with lung neoplasia and that this nucleic acid molecule is present in detectable levels in sputum specimens from patients with lung neoplasia. U.S. Patent No. 5,726,019 does not teach or suggest that the amount of mRNA encoding a catalytic subunit telomerase can be used to quantify tumor cells in a body fluid. U.S. Patent No. 5,726,019 does not teach or suggest anything regarding telomerase or mRNA coding for the catalytic subunit of telomerase. The GenBank submission, even assuming that it provides the sequence of the catalytic subunit of telomerase, provides no teaching or suggestion that the amount of mRNA present in a body fluid can be used to quantitate tumor cells. Furthermore, the Genbank submission does not teach or suggest the particular primers used in the instant methods or kits. Selby (GB 2 260 811) is directed to methods for diagnosing malignant tumors in which **total** mRNA is extracted from a sample of body fluid and reverse transcribed into cDNA, which is amplified using **primers** based upon **a tissue-specific gene not normally expressed in the body fluid**, followed by analysis to determine whether such amplified cDNA is present. Selby does not suggest using a gene for amplification that is not tissue specific nor does Selby suggest quantification of tumor cells in a body fluid. Selby, thus teaches using a tissue-specific gene, not a gene that is ubiquitously expressed. Further, Selby does not teach or suggest that the amount of the mRNA encoding the catalytic subunit of telomerase can be detected in a body fluid nor that such a amount is related to the number of tumor cells in the fluid.

Thus, none of the references, singly or in combination, teaches or suggests a method for the quantification of tumor cells in a body fluid by specifically amplifying the mRNA for the catalytic subunit of telomerase. Applicant's arguments were aimed at defining the special technical feature of the instant claims, and showing that because this special technical feature was not taught or suggested by any of the cited references or combinations thereof, the special technical feature of the instant pending and withdrawn claims was novel and inventive over the prior art. In the Requirement for Restriction set

3, 2001, the Examiner cited references that allegedly support (i) a Requirement for Restriction in the instant application; and (ii) an assertion that the special technical features linking either all the claims (allegedly "the mRNA for the catalytic subunit of telomerase"), the Group A claim set (allegedly "a method of amplification"), or the Group B claim set (allegedly a "method for tumor cell concentration") are all known in the prior art. In response, Applicant argued that (i) the special technical feature linking the instant pending and withdrawn claims is not "mRNA for the catalytic subunit of telomerase", but a method for the quantification of tumor cells in a body fluid and primers/kits used in said method; and (ii) that the special technical feature linking the instant pending and withdrawn claims is novel and inventive over the cited art. Applicant traversed the Examiner's Requirement for Restriction by pointing out the special technical feature of the claims, and by showing that, contrary to the Examiner's allegations, such special technical feature was not known in the art. Applicant's response was therefore commensurate with the Examiner's assertions in the Requirement for Restriction set forth on July 3, 2001.

(c) With regard to obviousness-type double patenting, the Examiner urges that claims of Group II are directed to a generic concentration method and the particulars of Group I would not be obvious over the generic method, unless each group of claims were amended to include particulars from the other, in which case obviousness-type double patenting would apply.

Applicant maintains that if the claims are divided into these groups, particularly Groups I and II, Applicant ultimately could be granted two patents, one directed to the method for quantifying tumor cells in a body fluid by concentrating or depleting tumor cells in a sample of a body fluid; specifically amplifying mRNA coding for the catalytic subunit of telomerase; and then quantitatively determining the amount of amplified nucleic acid to thereby quantifying tumor cells in a body fluid, and a second patent directed to the same method, but specifying that the cells are concentrated by layering the body fluid onto a cell separation medium and centrifuging the layered fluid and medium. If

the second patent, which is directed to claims that are encompassed within the claims of the first patent, were to issue first, obviousness-type double patenting **could not** be held. See MPEP 806, paragraph 3, which states:

> [w]here inventions are related as disclosed but are not distinct as claimed, restriction is never proper. Since, if restriction is required by the Office double patenting cannot be held, it is imperative the requirement should never be made where related inventions as claimed are not distinct.

See, also MPEP 804.01, which states:

> 35 U.S.C.121, third sentence, provides that wherein the Office requires restriction, the patent of either the parent or any divisional application thereof conforming to the requirement cannot be used as a reference against the other. This apparent nullification of double patenting as ground of rejection or invalidity in such cases imposes a heavy burden on the Office to guard against erroneous requirements for restriction where the claims define essentially the same inventions in different language and which, if acquiesced in, might result in the issuance of several patents for the same invention.

Contrary to the Examiner's urging that the Group II claims are directed to a "generic" concentration method, the Group II claims are in fact directed to the particulars of concentrating the tumor cells in the "Group I" method for quantifying the tumor cells in a body fluid by specifically amplifying the mRNA for the catalytic subunit of telomerase. In fact, as evidenced by the rejections under 35 U.S.C. §112, second paragraph and 35 U.S.C. §103(a) set forth and discussed below, the Examiner's examination of the Group II claims is based on incorporation of the particulars of Claim 1, a Group I claim into Claim 20, a Group II claim. Therefore, the Examiner requires incorporation of a Group I claim into a Group II claim, yet maintains that the Requirement for Restriction is proper and further adds that incorporation of Group I particulars into the Group II claims will result in the applicability of double-patenting if the Group II claims, which are encompassed within the method of the Group I claims, issues first. This is clearly in error. If examination of the Group II claims requires incorporation of the Group I particulars, then the restricted groups are <u>not</u>

patentably distinct, the Requirement should not be made, and, if made, double-patenting should not apply (MPEP 806, paragraph 3, *see* above).

In light of the above, Applicant filed a Petition under 37 C.F.R. §1.144 on December 26, 2001, requesting removal of the Restriction Requirement in the above-captioned application. Pending consideration of the aforementioned Petition, Applicant has retained the withdrawn Group I and Group III claims in addition to the pending Group II claims.

PRIORITY INFORMATION

The Examiner notes that an application in which the benefits of an earlier application are desired must contain a specific reference to the prior application(s) in the first sentence of the specification (37 C.F.R. §1.78). The specification has accordingly been amended to include such reference.

COMPLIANCE WITH SEQUENCE RULES

The Examiner alleges that the application fails to comply with the Rules for Nucleotide Sequences (MPEP 8.21-8.25) because (a) there is no hard copy of the Sequence Listing in the specification; and (b) the nucleotide sequences in the specification do not have SEQ ID. NOS.

With respect to (a), Applicant respectfully submits that both hard and disk copies of the Sequence Listing were filed August 4, 2000, in connection with the above-captioned application, together with a Preliminary Amendment, a Certified English Translation of the PCT application of which the instant application is a National Stage filing, a translated response to the Written Opinion, the International Preliminary Examination Report (IPER) and a Declaration for Patent Application. A copy of the date-stamped postcard indicating receipt of the aforementioned documents is attached to this Amendment. In addition, the Preliminary Amendment, which added claims 52-68, was entered into the instant application's file history, as evidenced by consideration of the added claims 52-68 in the Restriction Requirements and Office Action set forth in connection with the instant application. In the event that the originally filed hard and disk copies of the Sequence Listing have been

inadvertently misplaced or are otherwise missing, enclosed herein are duplicate hard and disk copies of the Sequence Listing originally filed August 4, 2000, in connection with the above-captioned application. With respect to (b), the specification has been amended so that the sequences in the specification now include reference to their corresponding SEQ ID. NOS. in the Sequence Listing. No new matter has been added.

THE REJECTION OF CLAIMS 20-34 and 62-66 UNDER 35 U.S.C. § 112, FIRST PARAGRAPH

Claims 20-34 and 62-66 are rejected under 35 U.S.C. §112, first paragraph, as allegedly containing subject matter that was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed subject matter. The Examiner alleges that the disclosure fails to describe how to quantify cells, and that absent a teaching of how to quantify tumor cells based on a quantity of mRNA for the catalytic subunit of telomerase, the disclosure fails to provide a written description and fails to demonstrate that Applicant was in possession of the claimed subject matter. This rejection is respectfully traversed.

Relevant Law

The purpose behind the written description requirement is to ensure that the patent applicant had possessio of the claimed subject mater at the time of filing of the application In re Wertheim, 541 F.2d 257, 262, 191 USPQ 90, 96 (CCPA 1976). The manner in which the specification meets the requirement is not material; it may be met by either an express or an implicit disclosure.

35 U.S.C. §112 requires a written description of the invention. This requirement is distinct from and not coterminous with the enablement requirement:

> The purpose of the 'written description' requirement is broader than to merely explain how to 'make and use'; the applicant must also convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention. The invention is,

> for purposes of the 'written description' inquiry, whatever is now claimed." Vas-Cath, Inc. v. Mahurkar, 935 F.2d at 1563-64, 19 USPQ2d at 1117 (emphasis in original).

The issue with respect to 35 U.S.C. §112, first paragraph, adequate written description has been stated as:

> [d]oes the specification convey clearly to those skilled in the art, to whom it is addressed, in any way, the information that appellants invented that specific compound [claimed embodiment] Vas-Cath, Inc. v. Mahurkar, at 1115, quoting In re Ruschig, 390 F.2d 1990, at 995-996, 154 USPQ 118 at 123 (CCPA 1967).

A specification must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention, *i.e.*, whatever is now claimed. Vas-Cath, Inc. v. Mahurkar, 935 F.2d 1555, 1563-64, 19 USPQ.2d 1111, 1117 (Fed. Cir. 1991). A written description requirement issue generally involves the question of whether the subject matter of a claim is supported by or conforms to the disclosure of an application as filed. The test for sufficiency of support in a patent application is whether the disclosure of the application relied upon "reasonably conveys to the artisan that the inventor had possession at that time of the later claimed subject matter." Ralston Purina Co. v. Far-Mar-Co., Inc., 772 F.2d 1570, 1575, 227 USPQ 177, 179 (Fed. Cir. 1985) (quoting In re Kaslow, 707 F.2d 1366, 1375, 217 USPQ 1089, 1096 (Fed. Cir. 1983)) (see also, MPEP 2163.02).

An objective standard for determining compliance with the written description requirement is "does the description clearly allow persons of skill in the art to recognize that he or she invented what is claimed." In re Gosteli, 872 F.2d 1008, 1012, 10 USPQ.2d 1614, 1618 (Fed. Cir.1989).

The Examiner has the initial burden of presenting evidence or reasons why persons skilled in the art would not recognize in an applicant's disclosure a description of the invention defined by the claims. In re Wertheim, 541 F.2d 257, 265, 191 USPQ 90, 98 (CCPA 1976); *See also* Ex parte Sorenson, 3 USPQ.2d 1462, 1463 (Bd. Pat.App. & Inter. 1987). By disclosing in a patent application a device that inherently performs a function or has a property,

operates according to a theory or has an advantage, a patent application necessarily discloses that function, theory or advantage, even though it says nothing explicit concerning it. The application may later be amended to recite the function, theory or advantage without introducing prohibited new matter. In re Reynolds, 443 F.2d 384, 170 USPQ 94 (CCPA 1971); and In re Smythe, 480 F. 2d 1376, 178 USPQ 279 (CCPA 1973).

Furthermore, the subject matter of the claim need not be described literally (*i.e.*, using the same terms or *inhaec verba*) in order for the disclosure to satisfy the description requirement. If a claim is amended to include subject matter, limitations, or terminology not present in the application as filed, involving a departure from, addition to, or deletion from the disclosure of the application as filed, the examiner should conclude that the claimed subject matter is not described in that application. This conclusion will result in the rejection of the claims affected under 35 U.S.C.112, first paragraph - description requirement, or denial of the benefit of the filing date of a previously filed application, as appropriate.

Analysis

The instant application describes methods for the specific detection and quantification of circulating tumor cells in body fluids before metastases become clinically manifest, or at an early stage of metastasis. As discussed below, it is respectfully submitted that the Examiner has provided no basis to doubt the veracity of what is claimed.

The claimed subject matter is directed to a method for the specific detection and quantification of circulating tumor cells by (a) concentrating the tumor cells from a sample of body fluid; (b) specifically amplifying, from the tumor cells, mRNA coding for the catalytic subunit of telomerase; and (c) quantitatively determining the amount of amplified nucleic acid, thereby quantifying tumor cells in the body fluid. The claims in the application as originally filed were directed to the aforementioned method and, therefore, by

definition, Applicant appreciated the claimed subject matter at the time of filing. The application further provides exemplary embodiments of each of the aforementioned steps (a) - (c).

As the Examiner has acknowledged, the application provides in great detail methods for concentrating tumor cells in blood, specifically amplifying mRNA for the catalytic subunit of telomerase and quantifying this mRNA by coamplification of a standard nucleotide sequence (pages 12-16, *e.g.*, and Examples). The application further describes how the concentrated tumor cell fraction can be enriched for telomerase-positive tumor cells while removing telomerase-positive non tumor cells by suitable purification, concentration and/or culturing techniques, described in the specification in great detail (pages 15-19, 24-25 and Figure 4, *e.g.*). Furthermore, contrary to the Examiner's assertion that there is no teaching in the specification of a correlation between the amount of mRNA for the catalytic subunit of telomerase and the quantity of tumor cells expressing the telomerase, the specification adequately describes such correlation.

For example, page 5, lines 16-25 of the specification provides that in spite of known methods for detecting tumor cells, there was a lack of "reliable and simple methods for quantifying tumor cells in a body fluid". The specification then goes on to recite that an object of the claimed subject matter is to provide a method to quantitate tumor cells in a body fluid. Further, page 26, lines 3-11 fo the specification provides that:

> Quantitative determination of the RNA component of telomerase in the sample **makes it possible to determine whether tumor cells, especially metastases, in particular micrometastases, of malignant tumors are present in the body fluid, and in what quantity.** This is of great use in particular for early clinical diagnosis of the formation of metastases from malignant tumors and for monitoring tumor therapy. (emphasis added)

Page 27, lines 4-5 of the specification describes that the claimed subject matter includes a kit for quantifying tumor cells in a body fluid. Further, the Figures and Examples in the specification provide detailed descriptions and

demonstrations of how a correlation may be established between the amount of amplified catalytic subunit of telomerase and the quantity of tumor cells. For example, Figure 5 of the specification shows a correlation between the amount of amplified product of the mRNA for the catalytic subunit of telomerase and the number of LNcap prostate carcinoma cells. Figure 5 demonstrates that the mRNA coding for the catalytic subunit of telomerase can be detected in as few as 10 cells. Figure 8 and the Examples section of the specification at page 32 demonstrates that amplification of the mRNA for the catalytic subunit of telomerase detects as few as 2 melanoma cells per ml of blood, when compared to blood that does not contain melanoma cells. Figure 9 and the Examples section of the specification at page 33, lines 3-21 shows a correlation between the amount of amplified mRNA for the catalytic subunit of telomerase and the quantity of prostate carcinoma cells or mamma carcinoma cells in blood. The results demonstrate that the method could be used to detect as few as 2 prostate carcinoma cells or 4 mamma carcinoma cells per ml of blood. Thus, there is ample description of a correlation between the amount of amplified mRNA for the catalytic subunit of telomerase and the quantity of tumor cells in a body fluid such as blood. Furthermore, it is not necessary to include in the specification that which those of skill in the art know. The specification is presumed to include all such knowledge. It is recognized and understood by those of skill in the art that when a particular gene in a cell is amplified and quantified, it is possible to deduce the number of cells therefrom. In particular, correlation of the amount of PCR-amplified nucleic acid with the number of cells was known those of skill in the art at the time of filing of the application (and well before).

To evidence this, exemplary publications describing the correlation between the amount of amplified nucleic acids and the quantity of cells from which the nucleic acids are amplified are attached hereto. For example, Mattano *et al.*, *Cancer Res.*, 52:4701-4705 (1992), attached hereto, discusses the sensitive detection of circulating neuroblastoma cells in peripheral blood by

Reverse Transcriptase - Polymerase Chain Reaction (RT-PCR) amplification of the neuroblastoma tumor marker, PGP 9.5. The Mattano article describes how RT-PCR of total RNA extracted from 10^6 peripheral blood cells spiked with serial dilutions of neuroblastoma tumor cells was used to determine a detection sensitivity, based on the detection of amplified PGP 9.5 product, of 1 neuroblastoma cell in 10^7 peripheral blood mononuclear cells (PBMNC). Poisson limiting dilution statistics, in which, for the same serial dilution, the proportion of reactions having no detectable tumor-specific RT-PCR amplification product is indicative of the number of tumor cells in the sample, was then used to verify the detection sensitivity. The Mattano article demonstrates that when the amount of total RNA used in a RT-PCR reaction is representative of a known number of (peripheral blood) cells, it is possible, using a combination of serial dilution and limiting dilution RT-PCR amplification of a neuroblastoma-specific gene product, to determine how many neuroblastoma tumor cells are present in a given population of normal peripheral blood cells.

Burchill *et al.*, *Int. J. Cancer*, 57:671-675 (1994), attached hereto, describes the detection of circulating neuroblastoma cells in peripheral blood by RT-PCR for tyrosine hydroxylase mRNA. The Burchill article discusses how the tyrosine hydroxylase RT-PCR amplified product could be detected starting with as little as 1-10 pg total mRNA from cultured neuroblastoma cells, potentially representing a single neuroblastoma cell (p. 672, column 2, third paragraph, Results Section). The amount of RT-PCR amplified product obtained starting with 1 pg to 100 ng total mRNA from the cultured neuroblastoma cells (positive controls, representing 1 to 10,000 neuroblastoma cells) was then compared against serial dilutions of 1 ml of blood spiked with neuroblastoma cells to arrive at a detection sensitivity of 1-10 neuroblastoma cells per ml of blood (approximately 10^7 blood cells) using this assay (p. 673, Figure 3). Further, the positive controls were compared against the RT-PCR amplification products of patient samples to detect evidence of circulating neuroblastoma cells and monitor the efficacy of tumor therapy (p. 674, Figures 4 and 5). Similar

experiments have shown the increase in band intensity of RT-PCR amplified CK 20 product as a function of the amount of RNA amplified from epithelial cancer cells, and of the number of epithelial cancer cells present in blood (Burchill *et al.*, *Br. J. Cancer*, 71:278-281 (February, 1995), attached hereto; *see* Figures 1c and 4). Other RT-PCR studies have quantitatively determined the sensitivity of detection of occult breast cancer cells and melanoma cells, showing that RT-PCR of keratin 19 (K19) transcripts can reliably detect 10 mammary carcinoma cells in 1 million normal peripheral blood mononuclear cells (Datta *et al.*, *J. Clin. Oncol.*, 12:475-482 (1994), attached hereto) and that RT-PCR of tyrosinase mRNA can detect as little as one melanoma cell per ml of blood (Foss *et al.*, *Br. J. Cancer*, 72:155-159 (July, 1995), attached hereto).

As demonstrated by the above exemplary publications, quantitation of cells from the amount of amplified RNA is routine, and, thus, it is not necessary to describe such method in detail in the specification. The failure to do so, certainly does not evidence that the inventors did not appreciate their discovery at the time of filing of this application.

Therefore, because there is adequate written description as to methods for concentrating tumor cells in blood, specifically amplifying mRNA for the catalytic subunit of telomerase, quantifying this mRNA by coamplification of a standard nucleotide sequence, and correlating the amount of amplified mRNA to the quantity of tumor cells, Applicant had possession of the claimed subject matter at the time of filing of the application.

THE REJECTION OF CLAIMS 20-34 AND 62-66 UNDER 35 U.S.C. §112, SECOND PARAGRAPH

Claims 20-34 and 62-66 are rejected under 35 U.S.C. 112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention. Various bases for this rejection are set forth and each is discussed in turn. Reconsideration of the grounds for rejection is respectfully requested in view of the amendments of the claims and the following remarks.

Relevant Law

Definiteness of claim language must be analyzed, not in a vacuum, but in light of (1) the content of the particular application disclosure, (2) the teachings of prior art, and (3) the interpretation claims would be given by one possessing the ordinary level of skill in the pertinent art at the time the invention was made. Claims need only "reasonably apprise those skilled in the art" of their scope and be "as precise as the subject permits." Hybritech Inc. v. Monoclonal Antibodies, Inc., 231 USPQ 81, 94 (Fed. Cir. 1986), cert. den., 480 U.S. 947 (1987). The Court in Orthokinetics, Inc v. Safety Travel Chairs, Inc., 1 USPQ2d 1081 (Fed. Cir. 1986) held that a claim limitation requiring that a pediatric wheelchair part be "so dimensioned as to be insertable through the space between the doorframe of an automobile and one of the seats" is definite. The Court stated:

> The phrase 'so dimensioned' is as accurate as the subject matter permits, automobiles being of various sizes. As long as those of ordinary skill in the art realized that the dimensions could be easily obtained, § 112, 2d ¶ requires nothing more. The patent law does not require that all possible lengths corresponding to the spaces in hundreds of different automobiles be listed in the patent, let alone that they be listed in the claims.

1 USPQ2d at 1088.

When one skilled in the art would understand all of the language in the claims when read in light of the specification, a claim is not indefinite.

Applicant is unaware of any requirement that terms be defined in the claims when one of skill in the art can readily determine the meaning of the term based on the description and definitions provided in the specification. In this respect, applicant is entitled to be its own lexicographer [see, e.g., MPEP 2111.01 "Applicant may be his or her own lexicographer as long as the meaning assigned to the term is not repugnant to the term's well known usage and utilize terms within the claims that are clear from a reading of the specification]. In re Hill, 73 USPQ 482 (CCPA 1947)". When applicant has

provided definitions in the specification, the claims are interpreted in light of such definition.

35 U.S.C. §112, second paragraph requires only reasonable precision in delineating the bounds of the claimed invention. The claim language is satisfactory if it reasonably apprises those of skill in the art of the bounds of the claimed invention and is as precise as the subject matter permits. Shatterproof Glass Corp.v. Libby-Owens Ford Col, 758 F.2d 613, 624, 225 USPQ 634, 641 (Fed. Cir), cert dismissed, 106 S. Ct. 340 (1985).

The amount of detail required to be included in the claims depends on the particular invention and the prior art and is not to be viewed in the abstract, but in conjunction with whether the specification is in compliance with the first paragraph of 35 U.S.C. §112. If the claims, read in light of the specification, reasonably apprise those skilled in the art of the utilization and scope of the invention, and if the language is as precise as the subject matter permits, the courts can demand no more:

> [i]t is not necessary that a claim recite each and every element needed for the practical utilization of the claimed subject matter (Bendix Corp. v United States, 600 F.2d 1364, 1369, 220 Ct. Cl. 507,514, 204 USPQ 617, 621 (1979); See, also, Carl Zeiss Stiftung v. Renishaw plc, 20 USPQ2d 1094, 1101).

Analysis

Item a) The Examiner alleges that the claims are indefinite in depending from a nonelected claim.

As discussed above, the nonelected claims, while withdrawn in light of the finality of the Requirement for Restriction, have been retained pending consideration of the Petition under 37 C.F.R. §1.144, filed December 26, 2001, for removal of the Restriction Requirement in the above-captioned application.

Item b) The Examiner alleges that the claims are incomplete in omitting essential steps, such omission amounting to a gap between the steps. The Examiner alleges that the omitted steps are: lysing the concentrated cells and isolating their RNA prior to amplification. This rejection is respectfully traversed.

As noted above, definiteness of claim language must be analyzed, not in a vacuum, but in light of (1) the content of the particular application disclosure, (2) the teachings of prior art, and (3) the interpretation claims would be given by one possessing the ordinary level of skill in the pertinent art at the time the invention was made. The instant claims are definite because the steps of (i) concentrating tumor cells; (ii) amplifying mRNA from the tumor cells; and (iii) quantitatively determining the amount of amplified mRNA to quantify the tumor cells, define the boundaries of the claimed subject matter in light of the disclosure in the specification and what is known to those of skill in the art.

Methods of amplifying mRNA from cells have long been known to those of skill in the art. As the specification describes and incorporates by reference, the amplification of mRNA from cells can be accomplished using a variety of standard techniques, such as are described in the specification and in the incorporated references. These standard techniques, known to those of skill in the art, include amplification of mRNA from crude cell lysates and/or RNA or other nucleic acids that are isolated from the cells after lysing the cells (see, *e.g.*, Nakamura *et al.*, Science, 277: 955-959 (1997); Meyerson *et al.*, Cell, 90: 785-795 (1997); Kim *et al.*, Science, 266:2011 (1994); International PCT Application Nos. WO 97/18322 and WO 96/01835; of record in the file history of the instant application, and pages 4-7 of the specification describing various embodiments of effecting such amplification). One of skill in the art, given the step of "amplification of mRNA of the catalytic subunit of telomerase from tumor cells", would recognize (i) the scope/boundaries of the claimed subject matter; and (ii) the standard techniques by which the amplification of mRNA from cells may be effected. No further elaboration of the amplification of mRNA from cells is necessary to render definite each of the steps of the claimed methods.

If the claims, read in light of the specification, reasonably apprise those skilled in the art of the utilization and scope of the invention, and if the language is as precise as the subject matter permits, the courts can demand no more:

> [i]t is not necessary that a claim recite each and every element needed for the practical utilization of the claimed subject matter (Bendix Corp. v United States, 600 F.2d 1364, 1369, 220 Ct. Cl. 507,514, 204 USPQ 617, 621 (1979); See, also, Carl Zeiss Stiftung v. Renishaw plc, 20 USPQ2d 1094, 1101).

It is respectfully submitted that in this instance, the claims reasonably apprise the skilled artisan of the scope and utilization of the claimed methods. The step of amplifying mRNA from tumor cells involves well-known methods that need not be elucidated for practical utilization of the claimed subject matter.

Item c) The Examiner alleges that the claims are confusing at the recitation "for concentrating the tumor cells" because it is unclear due to the improper claim format whether this is a method step.

This rejection has been obviated by amending Claim 20 to remove the phrase "for concentrating the tumor cells".

Item d) The Examiner alleges that the claims are further confusing because the method steps lack relationship with one another, *e.g.*, the source of the mRNA in step (b) is not stated.

This rejection has been obviated by amending step (b) to recite that the mRNA source is the tumor cells.

Item e) The Examiner alleges that Claim 24 has confusing syntax because the subject referred by "it" is unclear at both occurrences.

This rejection has been obviated by amending Claim 24 to recite that "it" is "body fluid".

Item f) The Examiner alleges that Claim 24 lacks proper antecedent basis because platelets are found in blood and Claim 20 does not recite that the body fluid is blood.

This rejection has been obviated by amending Claim 24 to recite that the body fluid is blood.

Item g) The Examiner alleges that Claim 29 is confusing and lacks proper antecedent basis in Claim 20 for the recitation "the tumor-cell-enriched

interphase" because the term "interphase", which is a stage in the cell cycle, is not related to the subject matter of Claim 20.

This rejection has been obviated by amending Claim 29 to recite "interface".

Item h) The Examiner alleges that the term "intensively" is a relative term, not adequately defined by the claim, which renders Claim 29 indefinite.

This rejection has been obviated by removing the term "intensively" from Claim 29.

Item i) The Examiner alleges that Claim 34 is confusing in lacking proper antecedent basis and being in improper claim format because no step of adding a dye to the cell separation medium is recited.

This rejection has been obviated by reciting the step of adding a dye to the cell separation medium.

Item j) The Examiner alleges that the claims are confusing because Claim 1, which is incoporated in Claim 20, has an improper "and" at the end of step (b).

As discussed above, Claim 1, although withdrawn, has been retained pending consideration of the Petition under 37 C.F.R. §1.144, filed December 26, 2001, for removal of the Restriction Requirement in the above-captioned application. The Examiner's objection has been addressed by amending retained Claim 1 to remove the "and" after step (b).

THE REJECTION OF CLAIMS 20-34 AND 62-67 UNDER 35 U.S.C. § 103(a)

Claims 20-34 and 62-67 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Selby (GB 2 260 811, April 28, 1993) in view of Nakamura *et al.* (Science, 277:955-959 (1997)) and further in view of Van Vlasselaer (5,648,223). The Examiner alleges that it would have been obvious to one of skill in the art to modify Selby, which allegedly teaches a method of concentrating tumor cells in a sample of body fluid and specifically amplifying an mRNA to detect tumor cells in the body fluid, with Nakamura, which allegedly teaches the amplification and quantification of mRNA for the catalytic subunit of

telomerase and a strong correlation, relative to the RNA component of telomerase, between increased telomerase activity and the mRNA for the catalytic subunit of telomerase (page 955, lines 10-17 and page 957, middle column), and further with Van Vlasselaer, which allegedly teaches a tumor cell concentration method employing a cell separation medium such as Percoll or Ficoll whose density is adjusted depending on the tumor cells to be concentrated, to obtain the subject matter of the instant claims. The Examiner alleges that it would have been further obvious to quantify the tumor cells by quantifying the mRNA for the catalytic subunit of telomerase in a test sample of the cells and comparing the ratio with that in the samples to be diagnosed as practised in the art.

The Examiner further alleges that with respect to several of the claims (23, 24, 62-64, 29, 30-34 and 65-67), it would have been *prima facie* obvious to: use Percoll and Ficoll as cell separation media that are "routinely used in the art" and also, *e.g.*, taught by Van Vlasselaer (Claim 23); provide a substance that prevents platelets from sticking to the tumor cells and to remove the platelets as "routinely practised in the art" (Claim 24); cool a sample after centrifugation as "routinely practised in the art" and as taught by Selby (Claim 29); use a barrier comprised of porous material, filter or sieve as "routinely practised" in conventional differential centrifugation where the material and the thickness and pore size thereof as well as the degree of dilution of the medium would have been selected according to experimental requirements, and employ a color separation medium for ease of layer recognition as "routinely practised in the art" (Claims 30-34 and 65-67). Reconsideration and withdrawal of this rejection is respectfully requested in view of the amendments herein and the following remarks.

Relevant law

In order to set forth a *prima facie* case of obviousness under 35 U.S.C. § 103: (1) there must be some teaching, suggestion or incentive supporting the combination of cited references to produce the claimed invention (*ACS Hospital*

Systems, Inc. v. Montefiore Hospital, 732 F.2d 1572, 1577, 221 USPQ 929, 933 (Fed. Cir. 1984)) and (2) the combination of the cited references must actually teach or suggest the claimed subject matter. Further, that which is within the capabilities of one skilled in the art is not synonymous with that which is obvious. *Ex parte Gerlach*, 212 USPQ 471 (Bd. APP. 1980). Obviousness is tested by "what the combined teachings of the references would have suggested to those of ordinary skill in the art" *In re Keller*, 642 F.2d 413, 425, 208 USPQ 871, 881 (CCPA 1981), but it cannot be established by combining the teachings of the prior art to produce the claimed subject matter, absent some teaching or suggestion supporting the combination (*ACS Hosp. Systems, Inc. v Montefiore Hosp.* 732 F.2d 1572, 1577. 221 USPQ 929, 933 (Fed. Cir. 1984)). "To imbue one of ordinary skill in the art with knowledge of the invention in suit, when no prior art reference or references of record convey or suggest that knowledge, is to fall victim to the insidious effect of a hindsight syndrome wherein that which only the inventor taught is used against its teacher" *W.L. Gore & Associates, Inc. v Garlock Inc.*, 721 F.2d 1540, 1553, 220 USPQ 303, 312-13 (Fed. Cir. 1983).

The prior art must provide a motivation whereby one of ordinary skill in the art would have been led to do that which the applicant has done. *Stratoflex Inc. v Aeroquip Corp.*, 713 F.2d 1530, 1535, 218 USPQ 871, 876 (Fed. Cir. 1983). In addition, the mere fact that the prior art may be modified in the manner suggested by the Examiner does not make the modification obvious unless the prior art suggests the desirability of the modification. *In re Fritch*, 23 USPQ 1783 (Fed. Cir. 1992).

Also, it is impermissible to ignore the advantages, properties, utilities and unexpected results that flow from the claimed invention; they are part of the invention as a whole. *In re Sernaker*, 702 F.2d 989, 217 USPQ 1 (Fed. Cir. 1983). Unexpected properties must always be considered when determining obviousness. A compound's structure and properties are inseparable so that

unexpected properties are part of the subject matter as a whole. *In re Papesh*, 315 F.2d 381, 137 USPQ 43 (CCPA 1963).

The Claims

Claim 20 is directed to the method of retained Claim 1 in which the step of concentration of tumor cells is effected by (i) covering a cell separation medium with a layer of the body fluid; (ii) centrifuging the cell separation medium covered with the body fluid; and (iii) collecting the tumor cells at the interface of the cell separation medium and the supernatant body fluid. Claims 21, 62 and 63 specify the density of the cell separation medium of Claim 20. Claim 22 specifies the time and speed of centrifugation, and Claim 23 specifies that the cell separation medium used is Percoll or Ficoll. Claims 25-27 specify that the body fluid is peripheral blood and further specify that the peripheral blood can be either arterial or venous blood and may be drawn in an anticoagulant substance and diluted with a diluent prior to covering the cell separation medium. Claim 64 is directed to the method of Claim 25 where the peripheral blood is drawn with an anticoagulant substance and, prior to covering the cell separation medium, diluted with a diluent at a ratio of about 1:1. Claim 24 specifies that the body fluid is blood and that prior to applying the sample to the cell separation medium, it is treated to prevent aggregation of platelets to tumor cells. Claim 28 specifies the types of body fluids that may be used in the method of Claim 20, and Claim 29 specifies that in the method of Claim 20, the centrifugation vessel is removed and cooled to prevent mixing of the cells. Claim 30 is directed to the method of Claim 20 wherein the centrifugation vessel is divided into two compartments by a porous barrier, filter or sieve, and the body fluid is introduced into the upper compartment. Claims 31, 32, 65 and 66 specify the pore size of the porous barrier, filter or sieve, and Claim 33 specifies that at least one of the porous barrier, filter or sieve is fabricated from or coated with a hydrophobic material. Claim 34 is directed to the method of Claim 20 in which a dye is added to the cell separation medium so as to distinguish the layer of cell separation medium from the layer of body fluid.

Claim 67 is directed to the method of Claim 1 where the tumor cells are derived from micrometastases of malignant tumors. Added Claims 69 and 70 specify that in the method of Claim 20, the tumor cells are separated from telomerase-positive non tumor cells.

Thus, all the claims include as one element, a method for the quantification of tumor in a body fluid. Further, all of the claims recite the steps of (i) concentrating the tumor cells; (ii) specifically amplifying, from the tumor cells, the mRNA for the catalytic subunit of telomerase; (iii) quantifying the amplified mRNA for the catalytic subunit of telomerase and correlating the quantity of amplified mRNA with the quantity of tumor cells in a body fluid. The dependent claims specify the particulars of concentration of the tumor cells, and Claim 67 specifies one source of tumor cells that may be used in the method. Added Claims 69 and 70 specify that the tumor cells are separated from the telomerase-positive non tumor cells.

None of the references teaches or suggests a method for the quantification of tumor cells in a body fluid. Therefore, a combination of the cited references cannot cure this deficiency. Further, none of the cited references, singly or in combination, lead to the combination of steps (i) - (iii) of the instantly claimed methods. Furthermore, none of the cited references, singly or in any combination, teaches or suggests separation of tumor cells from telomerase-positive non tumor cells.

Added Claims 69 and 70

Added Claims 69 and 70 are directed to the method of Claim 20 in which the tumor cells in the body fluid are separated from the telomerase-positive non tumor cells in the body fluid. As discussed below, not only do none of the cited references, singly or in any combination, teach or suggest a method for the quantification of tumor cells in a body fluid by specifically amplifying the mRNA for the catalytic subunit of telomerase, there is also no teaching or suggestion in any of the references or combinations thereof for the separation of tumor cells in a body fluid from telomerase-positive non tumor cells.

Teachings of the cited art, differences between the teachings of the cited art and rejected Claims 20-34 and 62-67, and differences between the teachings of the cited art and added Claims 69 and 70

Selby

Selby is directed to methods for diagnosing malignant tumors in which **total** mRNA is extracted from a sample of body fluid and reverse transcribed into cDNA, which is amplified using **primers** based upon **a tissue-specific gene not normally expressed in the body fluid**, followed by analysis to determine whether such amplified cDNA is present. Selby does not suggest using a gene for amplification that is not tissue specific nor does Selby suggest quantification of tumor cells in a body fluid. Selby thus teaches using a tissue-specific gene, not a gene that is ubiquitously expressed.

Further, Selby does not teach a method for concentrating or depleting tumor cells from a body fluid; Selby merely teaches the separation of plasma from all blood cells for isolating RNA from the blood cells (which may include tumor cells from a specific tissue that are then detected in a tissue-specific manner, as discussed above). Not only does Selby not teach or suggest the concentration of tumor cells from a body fluid, Selby does not teach or suggest any method for detecting all tumor cells in a body fluid, regardless of their cell type, much less that the level of of the catalytic subunit of telomerase, specifically amplified from concentrated tumor cells, is indicative of the presence of tumor cells in general in the body fluid.

Selby is directed to a method for detecting **a tissue-specific mRNA** in a sample using RT-PCR, and is of no relevance to the instantly claimed methods. Selby does not suggest anything about the catalytic subunit of telomerase nor suggest that it can be used for quantification of tumor cells in a sample of body fluid. Selby does not teach or suggest that the amount of the catalytic subunit of telomerase can be specifically amplified and quantitated in tumor cells concentrated or depleted from a body fluid, nor that such amount is related to the number of tumor cells in the fluid.

As discussed below, neither Nakamura *et al.*, which does not teach or suggest any diagnostic assay for the quantification of tumor cells in a body fluid, nor the use of specific amplification of the catalytic subunit of telomerase from tumor cells in such assay, nor Van Vlasselaer, which teaches neither an assay for the quantification of tumor cells in a body fluid, nor the use of the catalytic subunit of telomerase expression in such assay, nor concentration of tumor cells regardless of cell type, nor combinations of Nakamura *et al.* and Van Vlasselaer, can cure the deficiencies in Selby, which does not provide any teaching of the concentration of tumor cells from a body fluid and quantification of the tumor cells using a marker that is not tissue-specific.

Finally, with respect to added Claims 69 and 70, neither Selby nor, as discussed below, Nakamura *et al.* and Van Vlasselaer, have any teaching or suggestion of the separation of tumor cells in a body fluid from telomerase-positive non tumor cells.

Nakamura *et al.*

Nakamura *et al.* teaches the identification and characterization of the catalytic subunit of telomerase in fission yeast and human. Nakamura *et al.* teaches that the cataytic protein subunits of telomerase belong to the reverse transcriptase family, and are phylogenetically conserved.

Nakamura *et al.* teaches that the correlation between the abundance of the mRNA for the catalytic subunit of telomerase in various cell lines, and the telomerase activity in those cell lines, is stronger than the correlation between telomerase activity and telomerase RNA. **No body fluids** are tested; only immortal cell lines are tested for expression of the catalytic subunit of telomerase. Nakamura *et al.* does not teach, suggest or provide any indication as to whether the catalytic subunit of telomerase is expressed or not expressed in non tumor cells in body fluids. Further, Nakamura *et al.* does not teach or suggest quantification of tumor cells in a body fluid by any method, let alone specific amplification and quantitation of the catalytic subunit of telomerase in tumor cells, nor does it teach or suggest concentrating or depleting tumor cells

from a body fluid prior to such amplification. Furthermore, Nakamura *et al.* neither teaches nor suggests any diagnostic methods nor any methods for detecting tumor cells in body fluids, nor does it teach or suggest that the catalytic subunit of telomerase expressed by tumor cells would be specifically detectable, notwithstanding the presence of other body fluid cells that may express the catalytic subunit of telomerase, in a concentrated sample of tumor cells from body fluid. Even if Nakamura *et al.* teaches, as the Examiner alleges, a correlation between the abundance of mRNA of the catalytic subunit of telomerase and the telomerase activity of cells, there is no teaching or suggestion in Nakamura *et al.* regarding a method for quantifying circulating tumor cells in a body fluid, nor of specifically amplifying the mRNA for the catalytic subunit of telomerase from the tumor cells present in the body fluid. All samples tested by Nakamura *et al.* were cell lines, and Nakamura *et al.* provides no indication that tumor cells in a body fluid can be specifically detected and quantitated, notwithstanding other body fluid cells that may be present, by specifically amplifying, from tumor cells, the mRNA for the catalytic subunit of telomerase.

Nakamura *et al.* also does not teach or suggest that the amount of amplified catalytic subunit of telomerase can be used to quantify tumor cells in a sample of body fluid, nor does it teach or suggest the concentration of tumor cells in a body fluid. Nakamura *et al.* does not teach or suggest the separation of tumor cells from telomerase-positive non tumor cells, nor that the amount of the catalytic subunit of telomerase can be used to detect micrometastases of malignant tumors (Claim 67). Finally, Nakamura *et al.* neither teaches nor suggests any of the limitations of the rejected dependent claims discussed above.

Van Vlasselaer

Van Vlasselaer is directed to a method for enriching breast tumor cells from body fluids. Van Vlasselaer describes a method for concentrating breast cancer cells using a "cell-trap" centrifugation tube and a density gradient

solution such as percoll or Ficoll in which the density of the solution is specifically adjusted to enrich for the breast tumor cells, **depending on the specific type of breast tumor cell**. Van Vlasselaer does not teach any diagnostic assay for the detection or quantification of tumor cells **in general** in a body fluid. Further, Van Vlasselaer does not teach or suggest amplification and quantitation of the mRNA for the catalytic subunit of telomerase, or any other mRNA, for the quantification of tumor cells in a body fluid. Furthermore, in the concentration method of Van Vlasselaer, the density of the medium is adjusted **according to breast cancer cell type**. Van Vlasselaer does not teach or suggest a concentration step as claimed in the instant methods, where concentration of the tumor cells is effected **regardless of the tissue or cell type from which the circulating tumor cells originated**, and there is no teaching or suggestion in Van Vlasselaer of the separation of tumor cells from telomerase-positive non tumor cells.

Hence, as discussed below, the combination of teachings of Selby, Nakamura *et al.* and Van Vlasselaer are defective in failing to teach or suggest that (i) tumor cells can be concentrated from body fluid by a method which is independent of tumor cell type; (ii) the catalytic subunit of telomerase can be specifically amplified from the tumor cells regardless of the tissue of origin of the tumor cells, and regardless of the presence of non tumor cells in the body fluid; (iii) the separation of tumor cells from telomerase-positive non tumor cells; and (iv) the amount of amplified catalytic subunit of telomerase can be use for the quantification of tumor cells in a body fluid.

The combination of teachings of the cited references does not result in the instantly claimed methods

The combination of teachings of the cited references does not result in the instantly claimed methods. None of the cited references teaches or suggests any method for quantifying circulating tumor cells in a body fluid. None teach that tumor cells can be concentrated from the body fluid, regardless of tissue or tumor cell type, and that the catalytic subunit of telomerase can be

specifically amplified from the tumor cells concentrated or depleted from a body fluid.

With respect to added Claims 69 and 70, none of the references, singly or in combination, teaches or suggests the separation of tumor cells from telomerase-positive non tumor cells. Further, none teach or suggest that the specifically amplified catalytic subunit of telomerase can be correlated with the quantity of tumor cells in a body fluid. The cited references do not teach that the catalytic subunit of telomerase expressed by tumor cells in a body fluid may be specifically amplified in the presence of non tumor cells in a body fluid, nor whether non tumor cells in a body fluid express the catalytic subunit of telomerase. None teach or suggest that tumor cells in a body fluid can be specifically detected and quantitated, regardless of tissue type, by amplifying and quantitating the mRNA for the catalytic subunit of telomerase.

Thus, the cited references, singly or in any combination thereof, fail to teach or suggest the missing elements of the claims. Even if, as the Examiner alleges, Van Vlasselaer teaches the use of Percoll or Ficoll in centrifugation methods, Selby teaches cooling samples after centrifugation, and Nakamura *et al.* teaches a correlation between the expression of the catalytic subunit of telomerase and the level of telomerase activity in cell lines, none of these elements, singly or in combination, cure the deficiencies in the other teachings of the cited references that fail to provide a method for the quantification of tumor cells in a body fluid. None of the cited references, singly or in combination, provide any teaching or suggestion of a method for the quantification of tumor cells in a body fluid in which (i) the tumor cells are concentrated or depleted from the body fluid, regardless of cell type; (ii) the mRNA for the catalytic subunit of telomerase is specifically amplified from the tumor cells, regardless of their tissue of origin; and (iii) the amount of amplified mRNA of the catalytic subunit of telomerase can be correlated with the quantity of tumor cells in the body fluid. Further, with respect to added Claims 69 and 70, none of the cited references, singly or in any combination, provide any

teaching or suggestion of the separation of tumor cells from telomerase-positive non tumor cells.

Selby uses coamplification to detect and quantify tissue-specific genes, and Nakamura *et al.* and Van Vlasselaer do not provide any teaching for the detection and quantification of tumor cells, nor does Nakamura *et al.* provide a teaching as to the expression of the catalytic subunit of telomerase in any cells in a body fluid. Further, the concentration step of the instantly claimed methods, which concentrates the tumor cells from a body fluid regardless of their tissue and cell type, and which, in the case of added Claims 69 and 70, separates the tumor cells from the telomerase-positive non tumor cells, is not taught or suggested by the method in Selby, which merely separates plasma from blood, nor by the method in Van Vlasselaer, which teaches adjusting the density of the gradient solution according to the type of breast cancer cell, nor by Nakamura *et al.*, which does not teach or suggest any concentration step. Therefore, combinations of these references, all of which lack the elements of (i) concentrating tumor cells from a body fluid, regardless of tumor cell type; and (ii) specifically amplifying mRNA for the catalytic subunit of telomerase from tumor cells in a body fluid, regardless of tumor tissue type and regardless of whether other cells in body fluid may express the same gene, cannot cure these deficiencies with respect to the instant claims. None of the references or any combinations thereof teaches or suggests that the catalytic subunit of telomerase may be specifically amplified and quantified in tumor (as opposed to non-tumor) cells, and that such quantitation may be used to detect and quantify tumor cells in body fluids as a diagnostic assay.

As noted, Selby is directed to methods that require the use of a tissue-specific gene. Selby contemplates detection of a specific cancer based on a protein that is specifically expressed in the type of tissue from which the cancer originated. Selby does not teach or suggest an assay for the detection of tumor cells in a body fluid, regardless of cell type, and Selby certainly does not teach or suggest quantification of tumor cells. There are no teachings of record that

suggest that the catalytic subunit of telomerase is a tissue-specific gene. Moreover, Selby does not teach or suggest concentration of tumor cells from a body fluid; Selby merely separates plasma from the rest of the blood. Finally, with respect to added Claims 69 and 70, Selby does not teach or suggest separation of tumor cells from telomerase-positive non tumor cells.

Nakamura *et al.* is directed to the identification and characterization of the catalytic subunit of telomerase in fission yeast and humans. Nakamura *et al.* shows that the correlation of expression of mRNA for the catalytic subunit of telomerase with telomerase activity in various immortalized cell lines is stronger than the correlation of telomerase activity with telomerase RNA. Nakamura *et al.* does **not** show whether or not the catalytic subunit of telomerase can be detected in body fluids, much less that the catalytic subunit of telomerase can be specifically amplified from tumor cells in body fluids. Nakamura *et al.* does not suggest assays of any sort. Nakamura *et al.* does not teach or suggest concentration of tumor cells in a body fluid and, with respect to added Claims 69 and 70, there is not teaching or suggestion of separating tumor cells from telomerase-positive non tumor cells.

Van Vlasselaer also does not teach or suggest assays of any sort for the detection and quantification of tumor cells in general. Van Vlasselaer provides a method for the concentration of breast cancer cells from a body fluid, in which the density of the gradient is adjusted according to breast cancer cell type. Van Vlasselaer does not teach or suggest a general method for concentrating tumor cells in a body fluid, and Van Vlasselaer certainly does not use such concentration step in a method for the quantification of tumor cells in a body fluid by specific amplification of the catalytic subunit of telomerase. Further, with respect to added Claims 69 and 70, Van Vlasselaer does not teach or suggest separation of tumor cells from telomerase-positive non tumor cells. Hence, Van Vlasselaer does not cure the principal deficiencies in the teachings of Selby and Nakamura *et al.*

Hence the combinations of teachings fail to suggest several elements of the claimed methods, including but not limited to, concentration of tumor cells from a body fluid regardless of tumor cell type, and quantification of tumor cells in the body fluid by measuring the level of the catalytic subunit of telomerase specifically in the tumor cells.

Judicial Notice

Also, the Examiner cannot take judicial notice of facts outside the record that are not capable of instant and unquestionable demonstration. The Examiner makes quite a few such allegations. It is alleged that the follwing are obvious from what is "routinely practised in the art": 1) quantifying the tumor cells by quantifying the mRNA for the catalytic subunit of telomerase; 2) providing a substance that prevents platelets from sticking to tumor cells; 3) cooling samples after centrifugation; 4) using a barrier comprised of porous material, filter or sieve as routinely practised in conventional differential centrifugation wherein the material and the thickness and the pore size thereof as well as the degree of dilution of the medium can be selected according to experimental requirements; and 5) employing a colored separation medium for ease of layer recognition. No support for these allegations is provided, and none of the cited references teaches or suggests any of above elements.

The Examiner is reminded that MPEP 2144.03 states:

> The Examiner may take official notice of facts outside of the record which are capable of instant and unquestionable demonstration as being "well-known" in the art. In re Ahlert, 424 F.2d 1088, 1091, 165 USPQ 418, 420 (CCPA 1970). . . ..

The elements that the Examiner urges any one of ordinary skill in the art would have done in the context of the subject matter of this application are not "capable of instant and unquestionable demonstration as being "well-known" in the art. All of the above "routine art" has been shown to be applicable to the instantly claimed method for the quantification of tumor cells in a body fluid only through Applicant's own efforts. None of the cited references teaches or suggests, in a method for quantification of tumor cells in a body fluid, 1) a

correlation between the amount of mRNA for the catalytic subunit of telomerase and the quantity of tumor cells in a body fluid; 2) using a substance that prevents platelets from sticking to tumor cells before concentrating the tumor cells; 3) cooling centrifuged body fluid samples to separate layers of tumor cells from non tumor cells after centrifugation; 4) using a barrier comprised of porous material, filter or sieve with specified thicknesses or pore sizes according to the method of the instant claims; and 5) employing a colored separation medium for ease of layer recognition. Moreover, none of the cited references teach or suggest that the above "routine" practices would actually work in a method for the quantification of tumor cells in a body fluid.

In light of the scant teachings of relevance to the instant claims, evidence beyond official notice by the Examiner must be provided to establish that one of ordinary skill in the art would have been led to do what applicant has done:

> MPEP 2144.03 continues:
>
> If justified, the examiner should not be obliged to spend time to produce documentary proof. If the knowledge is of such notorious character that official notice can be taken, it is sufficient so to state. In re Malcolm, 129 F.2d 529, 54 USPQ 235 (CCPA 1942). If the applicant traverses such an assertion the examiner should cite a reference in support of his or her position.

In this instance, a reference or references supporting assertions by the Examiner should be provided. But even if such reference(s) were provided, it would not establish obviousness because, as discussed above, the principal elements of the claimed subject matter, namely, a method for the quantification of tumor cells in a body fluid by (i) concentrating the tumor cells, regardless of tumor cell type, from the body fluid; (ii) in the case of added Claims 69 and 70, separating the tumor cells from the telomerase-positive non tumor cells; (iii) specifically amplifying, from the tumor cells and regardless of its tissue type, mRNA for the catalytic subunit of telomerase; and (iv) quantitating the amplified

mRNA for the catalytic subunit of telomerase to thereby quantify the tumor cells in the body fluid; are lacking in all the cited references.

Therefore, the Examiner has failed to set forth a *prima facie* case of obviousness.

* * *

In view of the remarks herein, reconsideration of the requirement for restriction and examination of all claims on the merits are respectfully requested.

Respectfully submitted,
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